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Application No.:	10/616,082
Amendment Date:	22-Oct-08
Reply to Office Action of:	14 May 2008

REMARKS/ARGUMENTS

Claims 1-2, 6, 10-16, 18, 19, 26-30, and 57-58 are pending. Claims 3, 4, 5, 7, 8, 9, 17, 20-25, and 31-56 are cancelled.

Claims 1 and 2 have been amended to recite that the step comprises the step of expressing in the host cell a nucleic acid encoding a

chimeric mannosidase enzyme comprising

(a) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glc1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn6-s, and Mnn6-m or

(b) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glc1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m,

wherein said chimeric enzyme in (a) or (b) is capable of hydrolyzing *in vivo* more than 40% of the Man α -1,3 and/or Man α -1,6 linkages of a GlcNAcMan₅GlcNAc₂ substrate

Support for this amendment can be found in Table 11 of Example 14 and paragraph [698].

Additional support can be found in paragraphs [0062], [0063], and [0113]. Table 11 shows that particular chimeric mannosidase II enzymes when expressed in a *Pichia pastoris* host cell resulted in the cell being able to produce glycoproteins wherein at least 40% of the N-glycans had a GlcNAcMan₃GlcNAc₂ glycoform. The chimeric enzyme accomplished this by hydrolyzing the Man α -1,3 and Man α -1,6 linkages of the GlcNAcMan₅GlcNAc₂ N-glycans on the glycoprotein. Claims 1 and 2 claim only those particular chimeric mannosidase II enzymes.

Claim 10 has been amended to recite an embodiment of claim 1 or 2 wherein the chimeric enzyme comprises a Class IIx mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric enzyme to the secretory pathway of the host cell instead of the chimeric enzyme of Table 11. Support for the amendment can be found in for example paragraphs [0035] and [0057].

Claim 12 has been amended to recite an embodiment of claim 1 or 2 wherein the chimeric enzyme comprises a Class III mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric enzyme to the secretory pathway of the host cell

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instead of the chimeric enzyme of Table 11. Support for the amendment can be found in for example paragraphs [0039] and [0057].

These amendments are not believed to have introduced new matter into the application.

I. Claims 1, 2, 5-16, 18-30, 57, and 58 have been rejected under 35 U.S.C. § 112, second paragraph

Claims 1, 2, 5-16, 18-30, 57, and 58 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. In particular, the rejection states "In claims 1 and 2, initially it is claimed that an N-glycan comprising GlcNAcMan₅GlcNAc₂ is produced. Then later in the claim, an oligosaccharide substrate is used to produce a desired N-glycan, with the desired N-glycan having the oligosaccharide branch Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn. It is unclear whether the oligosaccharide branch Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn is added to GlcNAcMan₅GlcNAc₂, or whether it is added to a different substrate. If Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn is not added to GlcNAcMan₅GlcNAc₂, then it is unclear what purpose GlcNAcMan₅GlcNAc₂ has in the method."

Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn is the description of the oligosaccharide core structure of the product produced by taking GlcNAcMan₅GlcNAc₂ (the oligosaccharide substrate) and digesting it with a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Manα1,3 and Manα1,6 glycosidic linkage. Mannosidase II is such an enzyme. It can convert the oligosaccharide substrate GlcNAcMan₅GlcNAc₂ to the product GlcNAcMan₃GlcNAc₂. GlcNAcMan₃GlcNAc₂ comprises the Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn core structure. Figure 1B shows the conversion of GlcNAc₂Man₅GlcNAc₂ to GlcNAcMan₃GlcNAc₂. See also paragraph [0032] and Figure 36A.

Mannosidase IIx has an enzymatic activity similar to the enzymatic activity of mannosidase II but has a different substrate specificity. Mannosidase IIx can convert the oligosaccharide substrate Man₆GlcNAc₂ to the product Man₄GlcNAc₂. Man₄GlcNAc₂ comprises the Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn core structure. See also paragraph [0035] and Figure 36B.

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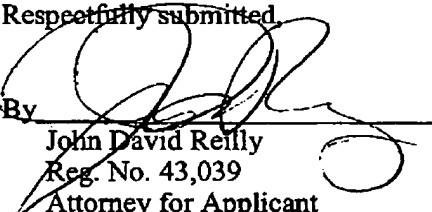
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Mannosidase III has an enzymatic activity similar to the enzymatic activities of mannosidases II and IIx but has a substrate specificity that differs from both mannosidase II and mannosidase IIx. Mannosidase III can convert the oligosaccharide substrate Man₅GlcNAc₂ to the product Man₃GlcNAc₂. Man₃GlcNAc₂ has the Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn core structure. See also paragraph [0039] and Figure 36C.

The claims have been amended to help clarify what the method entails. In light of the above, reconsideration of the rejection is requested.

CONDITIONAL PETITION

Applicant hereby makes a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

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